

Figure1
Rat Nedd4

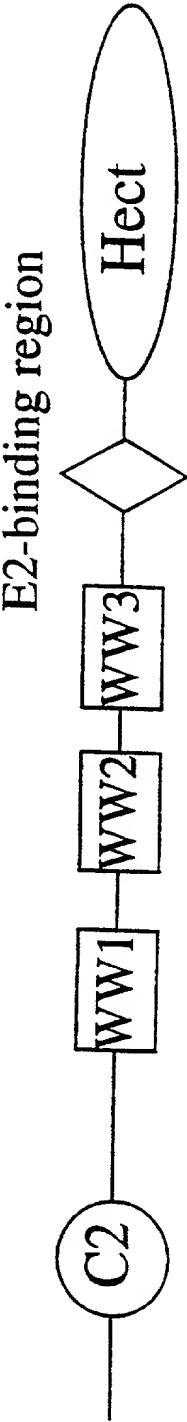


Figure 2: Clone 7.7 is the homolog of human clone KIAA0313

- Clone 7.7 exhibits 75% identity and 95% similarity of human clone KIAA0313.

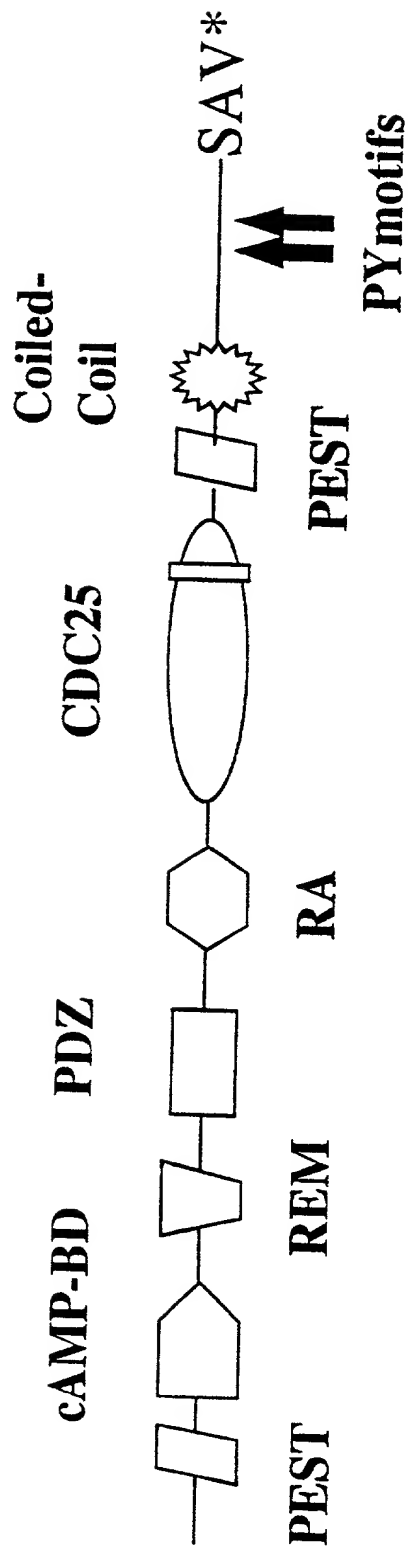
Clone7.7
KIAA0313

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GGKDVSIEAESSSLTSVTTEETKPVPMPAHIAVASSTTKGLIARKEGRYREPPPTPPGYI
***** *****:..*****,*****; *****; *****;

Clone7.7
KIAA0313
GIPIADFEGCHPARKPPDYNVALQSRMVARPTEAPAG--QTPP-AAAAARPGSKPQ
GIPITDFEGHSHPARKPPDYNVALQSRMVARSSDTAGPSSVQQPHGHPTSSRPVNKPQ
:*.*****:::***. * * * * * : * * * * *

Clone7.7
KIAA0313

Figure 3A Schematic Diagram of GRF4



4618 b.p.

1/1 31/11
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 M K P L A I P A N H G V M G Q Q E K H S
 61/21 91/31
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 L P A D F T K L H L T D S L H P Q V T H
 121/41 151/51
 gtt tct tct agc cat tca gga tgt agt atc act agt gat tct ggg agc agc agt ctt tct
 V S S S H S G C S I T S D S G S S S L S
 181/61 211/71
 gat atc tac cag gcc aca gaa agc gag gct ggt gat atg gac ctg agt ggg ttg cca gaa
 D I Y Q A T E S E A G D M D L S G L P E
 241/81 271/91
 aca gca gtg gat tcc gaa gac gac gat gaa gaa gac att gag aga gca tca gat cct
 T A V D S E D D D E E D I E R A S D P
 301/101 331/111
 ctg atg agc agg gac att gtg aga gac tgc cta gag aag gac cca att gac cgg aca gat
 L M S R D I V R D C L E K D P I D R T D
 361/121 391/131
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 D D I E Q L L E F M H Q L P A F A N M T
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 601/201 631/211
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 661/221 691/231
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 C Q F V C I A Q O D Y C R I L N Q V E K
 721/241 751/251
 aac atg caa aaa gtt gaa gag gaa gga gag att gtt atg gtg aaa gaa cac cga gaa ctt
 N M Q K V E E E G E I V M V K E H R E L
 781/261 811/271
 gat cga act gga aca aga aag gga cac att gtc atc aag ggt acc tca gaa agg tta aca
 D R T G T R K G H I V I K G T S E R L T
 841/281 871/291
 atg cat ttg gtg gaa gag cat tca gta gta gat cca aca ttc ata gaa gac ttt ctg ttg
 M H L V E E H S V V D P T F I E D F L L
 901/301 931/311
 acc tat agg act ttt ctt tct agc cca atg gaa gtg ggc aaa aag tta ttg gag tgg ttt
 T Y R T F L S S P M E V G K K L L E W F
 961/321 991/331
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 N D P S L R D K V T R V V L L W V N N H
 1021/341 1051/351
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 F N D F E G D P A M T R F L E E F E N N

cNMP-BD

REM

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Figure 3B

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1081/361 1111/371
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L E R E K M G G H L R L L N I A C A A K
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A K R R L M T L T K P S R E A P L P F I
 1201/401 1231/411
 tta ctt gga ggc tct gag aag gga ttt gga atc ttt gtt gac agt gta gat tca ggt agc
L L G G S E K G F G I F V D S V D S G S
 1261/421 1291/431
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 1321/441 1351/451
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F E N I Q L S K A M E I L R N N T H L S
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I T V K T N L F V F K E L L T R L S E E
 1441/481 1471/491
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K R N G A P H L P K I G D I K K A S R Y
 1501/501 1531/511
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S I P D L A V D V E Q V I G L E K V N K
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 cgg atc agt atc ttg cca cag aaa cca tac aat gat att ggg att ggt cag tct caa gat
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T L S S S N P D L L Q S H H R I L D F S
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Y I M I S K D T T A K E V V I Q A I R E
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F A V T A T P D Q Y S L C E V S V T P E
 1981/661 2011/671
 gga gta atc aaa caa aga aga ctt cca gat cag ctt tcc aaa ctt gca gac aga ata caa
G V I K O R R L P D O L S K L A D R I Q
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D A Q E L L R E S Q I S L L Q L S T V E
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 gtt gca aca cag ctc tct atg cga aat ttt gaa ctc ttt cgc aac att gaa cct act gaa
V A T O L S M R N F E L F R N I E P T E

PDZ

RA

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C K N F N S M F A I I S G L N L A P V A
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Q D L F D P S R N M A K Y R N V L N S Q
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N L Q P P I I P L F P V I K K D L T F L
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H E G N D S K V D G L V N F E K L R M I
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 2761/921 2791/931
 ttc agg act cgg aag aag aaa tgg cgg agt ttg ggg tct ctc agc cag ggt agt aca aat
F R T R K K K W R S L G S L S Q G S T N
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P Q A L K K I L S L S E E G S L E R H K
 3301/1101 3331/1111
 aaa cag gct gaa gat aca ata tca aat gca tct tgg cag ctt tct tct cct cct act tct
K Q A E D T I S N A S S Q L S S P P T S

CDC25

Insertion
Unique to
GRF4

Continuation of the
CDC25 domain

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3361/1121 3391/1131
 cca cag agt tct cca agg aaa ggc tat act ttg gct ccc agt ggt act gtg gat aat ttt
 P Q S S P R K G Y T L A P S G T V D N F
 3421/1141 3451/1151
 tca gat tct ggt cac agt gaa att tct tca cga tcc agt att gtt agc aat tcg tct ttt
 S D S G H S E I S S R S S I V S N S S F
 3481/1161 3511/1171
 gac tca gtg cca gtc tca ctg cac gat gag agg cgc cag agg cat tct gtc agc atc gtg
 D S V P V S L H D E R R Q R H S V S I V
 3541/1181 3571/1191
 gaa aca aac cta ggg atg ggc agg atg gag agg cgg acc atg att gaa cct gat cag tat
 E T N L G M G R M E R R T M I E P D Q Y
 3601/1201 3631/1211
 agc ttg ggg tcc tat gca cca atg tcc gag ggc cga ggc tta tat gct aca gct aca gta
 S L G S Y A P M S E G R G L Y A T A T V
 3661/1221 3691/1231
 att tct tct cca agc aca gag gaa ctt tcc cag gat cag ggg gat cgc gcg tca ctt gat
 I S S P S T E E L S Q D Q G D R A S L D
 3721/1241 3751/1251
 gct gct gac agt ggc cgt ggg agc tgg acg tca tgc tca agt ggc tcc cat gat aat ata
 A A D S G R G S W T S C S S G S H D N I
 3781/1261 3811/1271
 cag acg atc cag cac cag aga agc tgg gag act ctt cca ttc ggg cat act cac ttt gat
 Q T I Q H Q R S W E T L P F G H T H F D
 3841/1281 3871/1291
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 Y S G D P A G L W A S S S H M D Q I M F
 3901/1301 3931/1311
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 3961/1321 3991/1331
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 Q S R A S W A S S T G Y W G E D S E G D
 4021/1341 4051/1351
 aca ggc aca ata aag cgg agg ggt gga aag gat gtt tcc att gaa gcc gaa agc agt agc
 T G T I K R R G G K D V S I E A E S S S
 4081/1361 4111/1371
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 4141/1381 4171/1391
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 A S S T T K G L I A R K E G R Y R E P P
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 4321/1441 4351/1451
 tcc tcc gac aca gct ggg cct tca tcc gta cag cag cca cat ggg cat ccc acc agc agc
 S S D T A G P S S V Q Q P H G H P T S S
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 R P V N K P Q W H K P N E S D P R L A P

PY motifs

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4441/1481

4471/1491

tat cag tcc caa ggg ttt tcc acc gag gag gat gaa gat gaa caa gtt tct gct gtt tga
Y Q S Q G F S T E E D E D E Q V S A V *

4501/1501

4531/1511

PDZ binding motif

ggc aca gac ttt tct gga agc aga gcg agc cac ctg aaa gga gag cac aag aag acg tcc
G T D F S G S R A S H L K G E H K K T S

4561/1521

4591/1531

tga gca ttg gag cct tgg aac tca cat tct gag gac ggt gga cca gtt tgc ctc ctt c
* A L E P W N S H S E D G G P V C L L

CDC25 Domain

hGRF4 SQISLLQLSTVEVATQLSMRNFELFRNIEPTEYIDDLF---KLRSKTSCANLKRFEVIN
dGRF4 SNVHFLHLNAYELAIQTLTQDFANFRQIESTEYVDELF---ELRSRYGVPMLSKEAELVN
hEpac SAEGLDLVSADLAGQLTDHDSLFNSIHQVELIHYVLGPOHLRDVT-TANLERFMRRFN
mRasGRF2 KAECFETLSAMELAEQITLLDHIVFRSIPYEEFLGQGW--MKLDKNERTPYIMKTSQHFN
dSOS DEITLLTLHPLELARQLTLLEFEMYKNVKPSELVGSPT--KKDKVKSPNLLKMKHTT
hRasGRP VSLFDHLEPEELSEHLTYLEFKSFRRISFSQYQNYLVN----SCVKENPTMERIALCN
: : . : : : : : : : : : . : : .

hGRF4 QETFWVASEILRETQNLKRMKIIKHFIKIALHCRECKNFNSMFIIISGLNLAPVARLRTT
dGRF4 REMFWVSEICAENIVRRMKIVQFIKIARHCKEKNFNSMFIVSGLGHGAVSRLRQT
hEpac ELQYWVATELCCLPVPGPRAQLLRKFIKLAHLKEQKNLSFFAVMFGLSNSAISRLAHT
mRasGRF2 EMSNLVASQIMNYADISSRPNAIEKWAVADICRCLHNYNGVLEITSALNRSPIYRLKKT
dSOS NVTRWIEKSITEAENYEERLAIMQRAIEVMMVMLELNNFNGLSIVAAMGTASVYRLRWT
hRasGRP GISQWVQLMVLRSPTQLRAEVFIKFIQVAQKLHQLQNFNTLMAVIGGLCHSSISRLKET
: : * : : : . * * : : . : . : * *

hGRF4 WEKLPNKYEKLFQDLQDLFDPSSRNMAKYRNVLSQNL-QPPIIPLFPVIKKDLTFLEHGN
dGRF4 WEKLPISKYQRLFNDLQDLMDPSRNSKYRQLVSAELLAQHPIIPFYPIVKKDLTFIHLGN
hEpac WERLPHKVRKLYSALERLLDPSWNHRVYR-LALAKLS--PPVIPFMPLLLKDMTFIHEGN
mRasGRF2 WAKVSKQTKALMDKLQKTVSSEGRFKNLR-ETLKNCN--PPAVPYLGMYLTDLAFIEEGT
dSOS FOGLPERYRKFFLEECRELSDDHLKKYQER--L-RSIN--PPCVPPFGRYLTNHLHEEGN
hRasGRP SSHVPHEINKVLGEMTELLSSSRNYDNYR-RAYGECT--DFKIPILGVHLKDLISLYEAM
: * . : * : : .

hGRF4 DSKVDG--LVNFEKLRMIAKEIRHVGRMASVNMDPALMFTRKKK----WRSLSLSQGS
dGRF4 DTRVDG--LINFELRLMLAKEVRLTHMCSSPYDLLSILELKGQSPSNALFSLNQMSASQ
hEpac HTLVEN--LINFELKMRMMARAARMLHCRSHNPVPLSPLRSRVSHLHEDSQVARISTCSE
mRasGRF2 PNFTEEG-LVNFSKMRMISHIIREIRQFQQTAYRIDQQP-----
dSOS PDLLANTELINFSKRRKVAEIIIGEIQYQYQNPYCLNEES-----
hRasGRP PDYLEDG-KVNVHKLALYNHISELVQLQEVAPPLEANKDL-----
: * . : : .

hGRF4 TNATVLDVAQTGG----HKKRVRSSFLNAKKLYEDAQMARKVKQYLSNLELEMDDESLQ
dGRF4 SNAAAGTVIAANAGQATIKRRKKSTAAPNPKMFEEAQMVRRVKAYLNSLKILSDEDLLH
hEpac -----QSLSTRSPASTWAYVQQLKVIDNQRELS
mRasGRF2 -----KVIQYLLDKALVIDEDSLY
dSOS -----TIRQFFEQLDPFNGLSDKQMSDYLY
hRasGRP -----VHLLTSLDLYYTEDEIY
. :

Figure 4

hGRF4 TSLQCEPATNT
dGRF4 KFSLECEPAHGS
hEpac RLSRELEP----
mRasGRF2 ELSLKIEPRLPA
dSOS NESLRIEPRGCK
hRasGRP ELSYAREPRNHR
* **

Figure 5: GRF4-REM domain

CDC25	-IRGGTKEALIEHLT-SHELVDAAFNVMTLITFRSILT-TREFFYALIYRY-
Sos_mouse_	-IKGGTVVKLIERLT-YHMYADPNF-VRTFLTYSFCK-PQELLNLLIERFE
RasGEF_aimless_	VVKFASLNKLVEHLT-HDSKHDLQFLKTFMLTYQSFCF-PEKLSKLLQORY-
GRF2_mouse_	-IRYASVEALLERLT-DLRFSLIDFLNTFLHTYRIFTT-ATVVLAKLSDIY-
GRF4	-IKG-TSERLTMHLVEHSVVDPTFIEDFLTYRTFLSSPMEVGKLLLEWFN
	:: : * : * . . * : : : . . . * :

Figure 6A:

Overall structure comparison between
GRF4 and other known mammalian

RasGEF/RasGRF

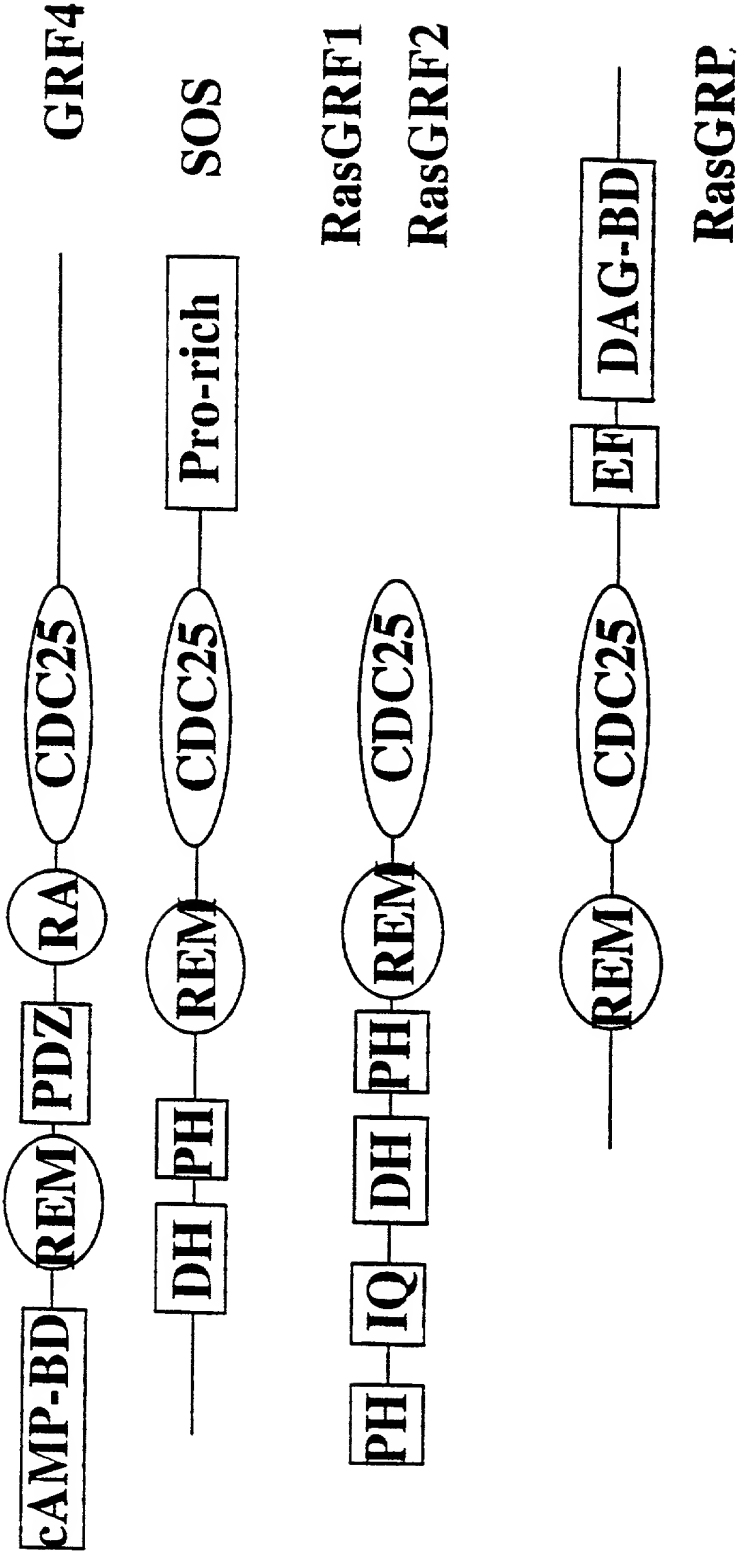
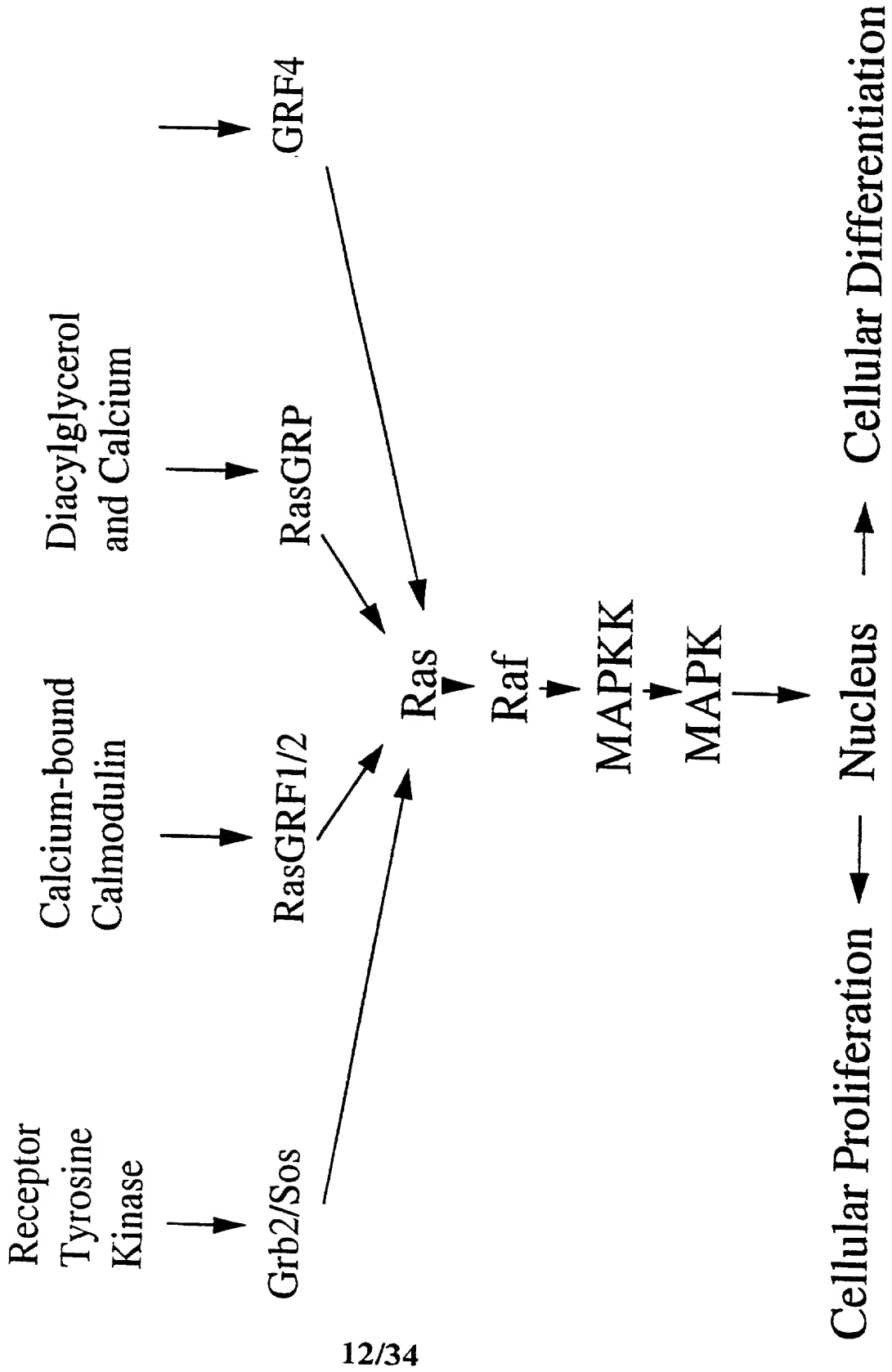


Fig.6B

Ras Signaling pathway



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100220" 92874660

Figure 7

PDZ domain

hGRF4	LTKPSREAPLPFILLGSEK-----GFGIFVDSVDSGSKATEAG-LKRGDQIL
dGRF4	LTRSSRDEPLNFRIVGGYELRGVAIATGNAAVGIYISHVEPGSKAQDVG-LKRGDQIH
hPTP-BAS-1	NLKKDAKYGLGFQIIGGEKMGRL-----DLGIFISSVAPGPGPADLDGCLKPGDRLI
hPSD-95	IVIHRGSTGLGFNIVGGEDG-----EGIFISFILAGGPADLSGELRKGDQIL
rLin-7-C	VELPKTEEGLGFNIMGKEQ-----NSPIYISRIIPGGIADRHGGLKRGDQLL
hDGL	VKVQKGSEPLGISIVSGEKG-----GIYVSKVTVGSIHAHQAG-LEYGDQLL
	. * : : : * . * : : : * : : : * : : : * : : : *
hGRF4	EVNGQNFENIQLSKAMEILR
dGRF4	EVNGQSLDHVTSKRALEILT
hPTP-BAS-1	SVNSVSLEGVSHHAAIEILQ
hPSD-95	SVNGVDLNRNASHEQAAIALK
rLin-7-C	SVNGVSVEGEHHEKAVELLK
hDGL	EFNGINLRSATEQQARLIIG
	..* . . * :

cNMP-BD

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Figure 9:

GRF4-RA domain

dgk-1a_ce_ GRF4 RalGDS_h_	<pre> -----REDFE---IIRVFDGNN---YRSQIS-----RNIIVAKHVSQQVRDAALR --HHRILDFS---ATPDLPDQVLRFKADQOS-----RYIMISKDTTAKEVVIQAIR SILVTSQDKAPSVISRVLKKNNRDSAVASEYELVQLLPGERELTIPASANVFYAMDGASH * : : : .: . * : : . . . * :</pre>
dgk-1a_ce_ GRF4 RalGDS_h_	<pre> RFHI--NDTPERYIT-QVVGEVEEEILED-----PVP-----LRNVKRPEGKRAQIFIR EFAV--TATPDQYSLC-EVSVTPEGVIKQ-----RLP-----DQLSK--LADRIQLSGR DFLLRHGEGPLLLHLASPVARLPQELLRVREEGAPFPGSRPQGGRLHGHCSSEEEAPLAYR * : * : * : : * . * : : *</pre>
dgk-1a_ce_ GRF4 RalGDS_h_	<pre> YVD----- YYLKNNME- SHGVHTRCG :</pre>

Figure 10

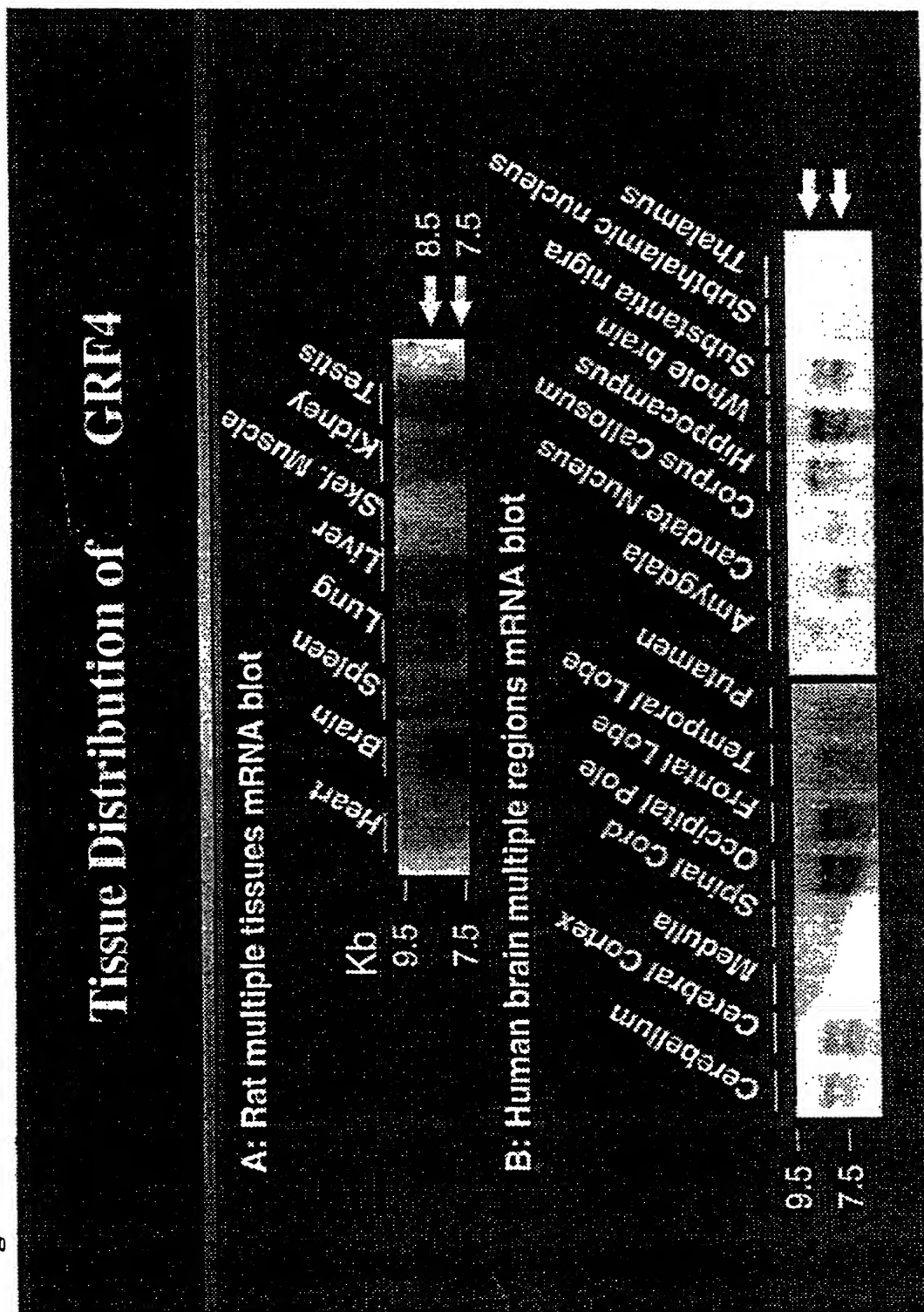


Figure 11

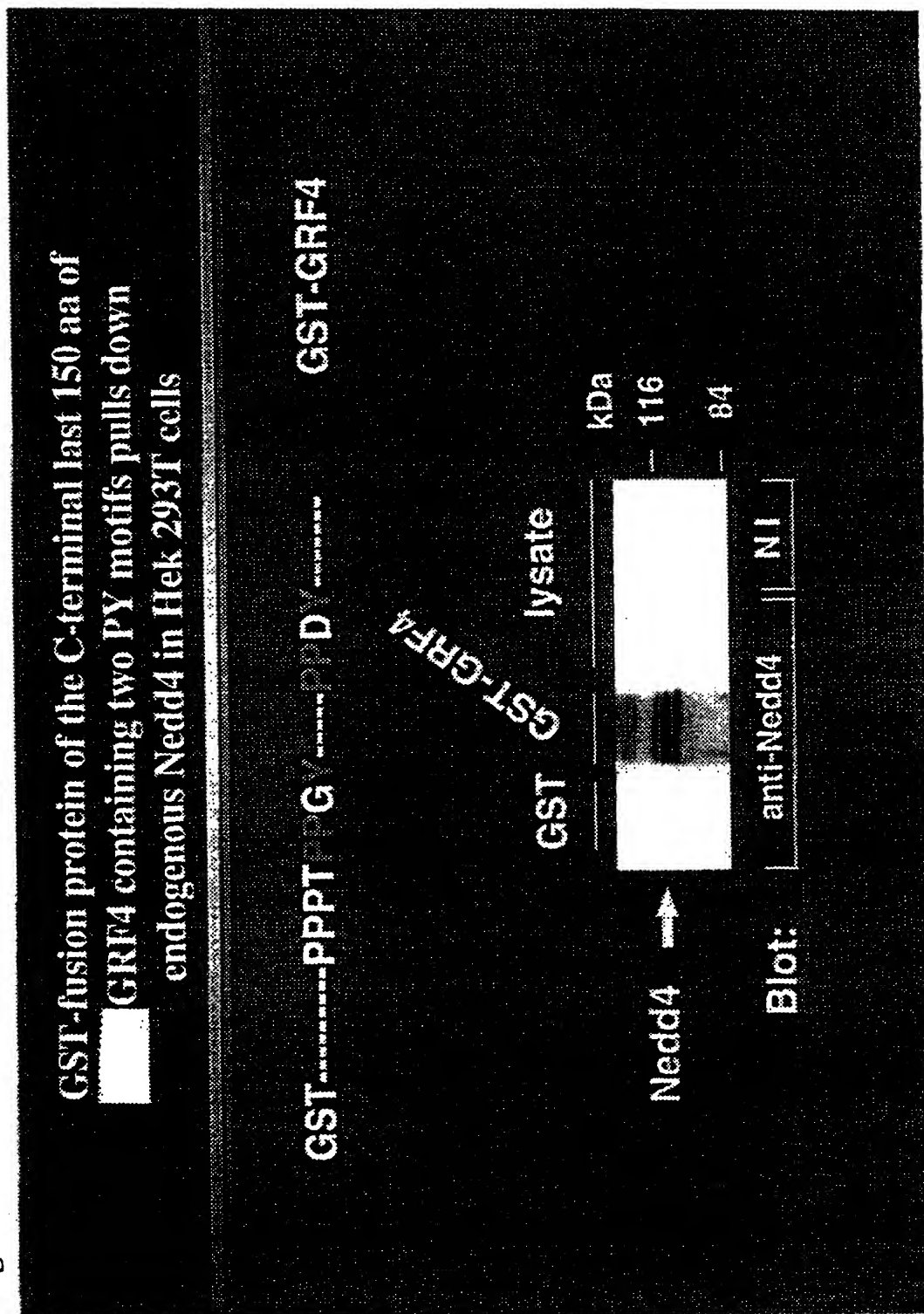


Figure 12

GRF4 co-immunoprecipitates with endogenous Nedd4 in Hek 293T cells transiently transfected with Flag-tagged GRF4

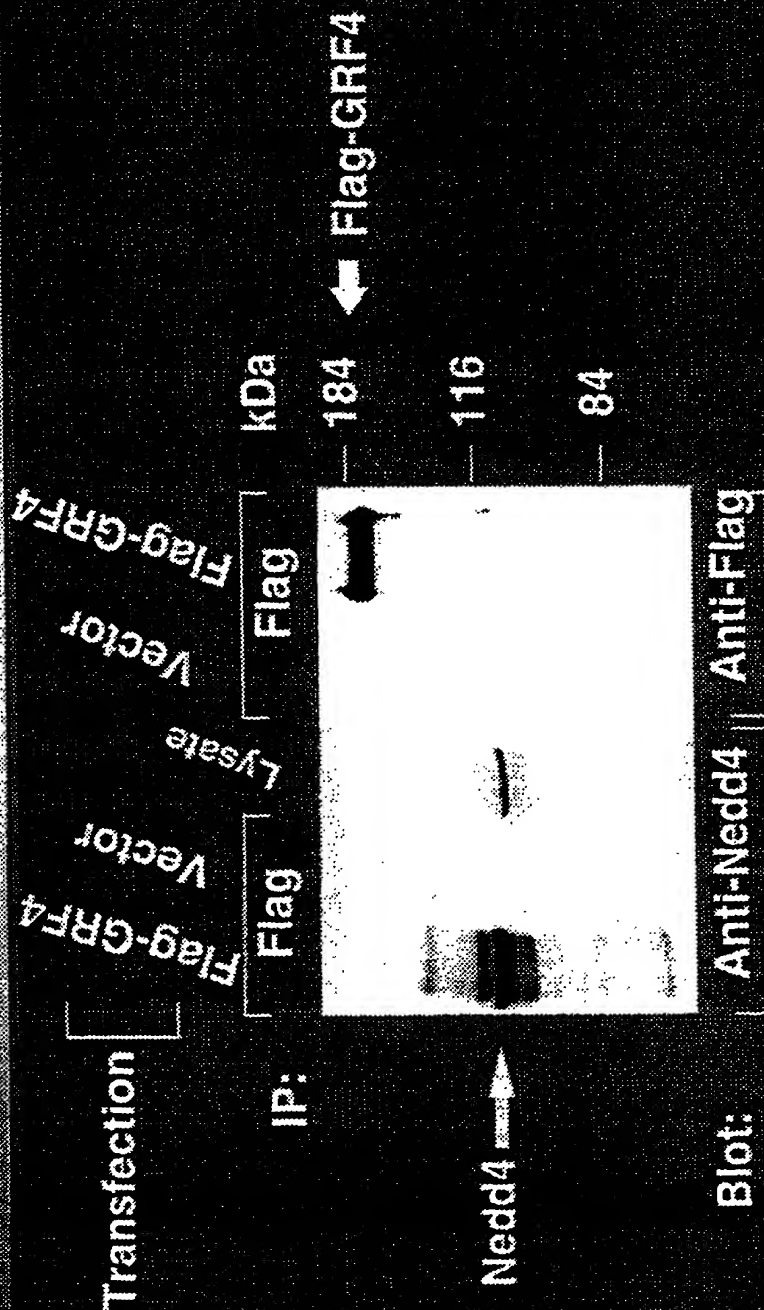


Fig.13

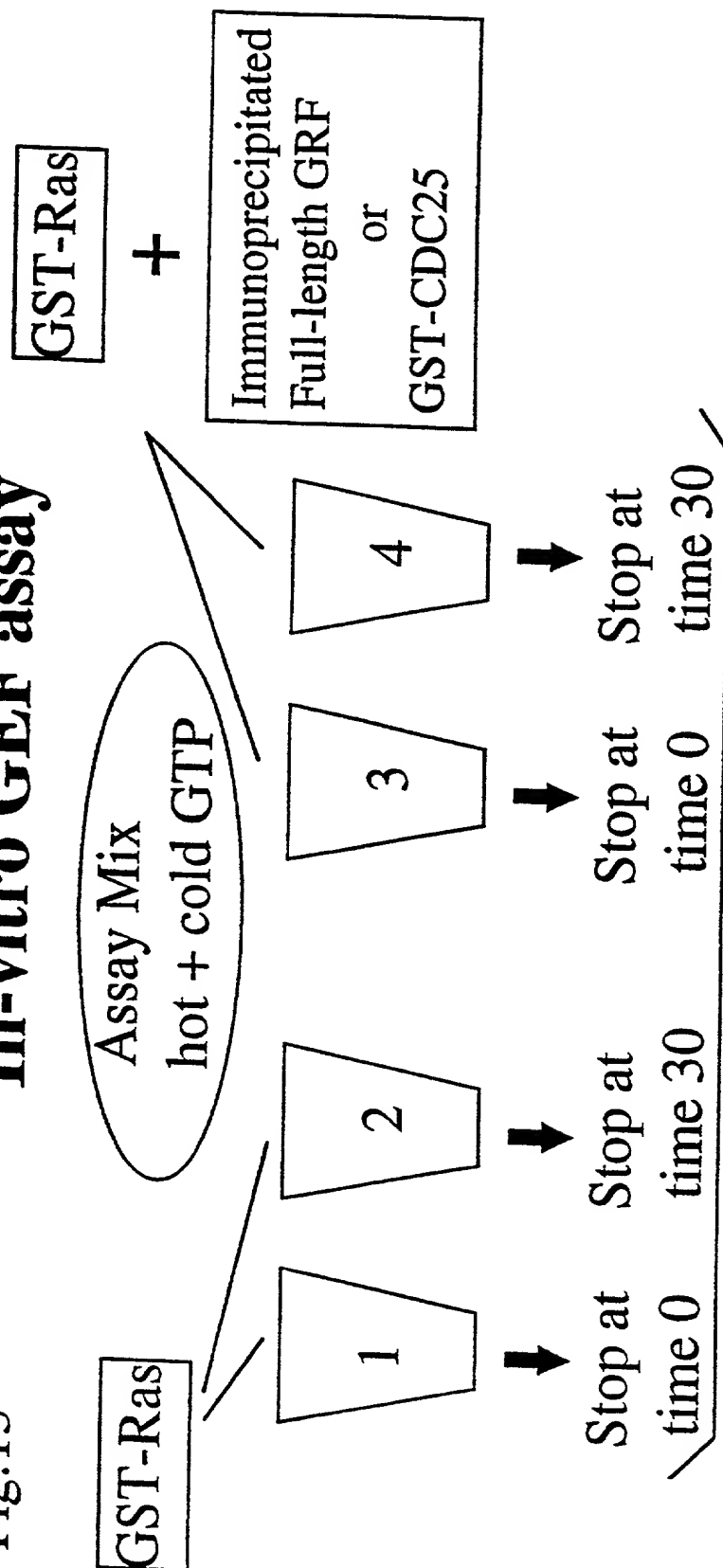
In-vitro GEF assay

Fig.14 GRF4 is active on Ras : in-vitro GEF
assay using immunoprecipitated full-length
GRF4

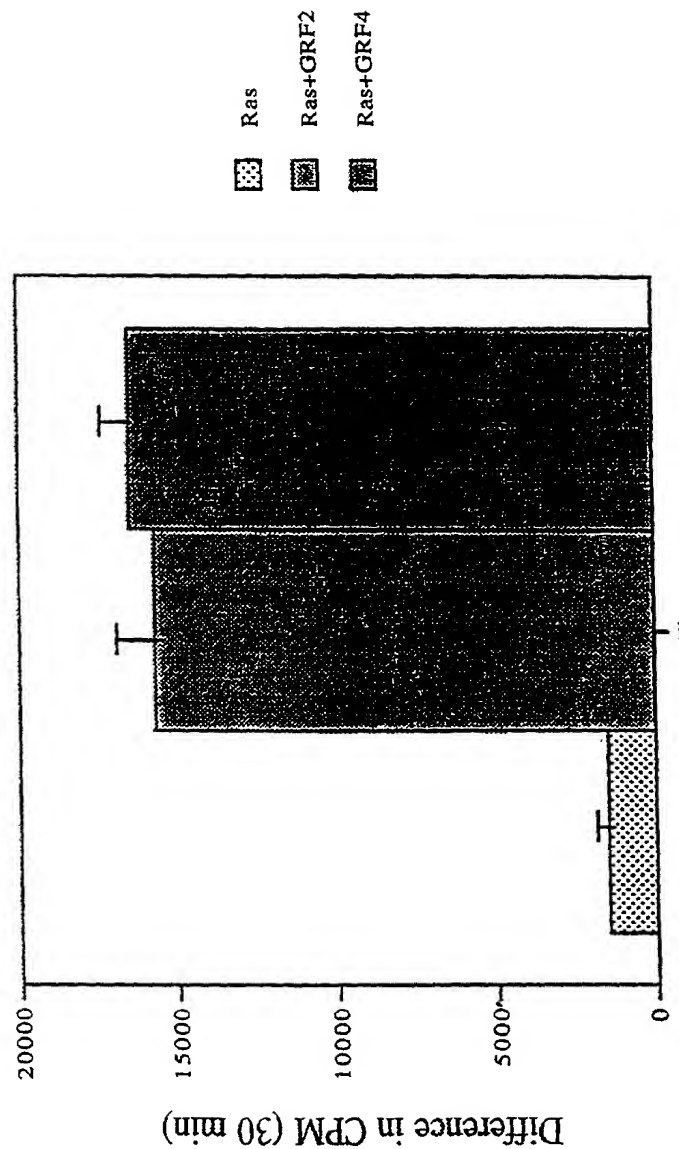


FIGURE 15

Figure 15

GRF4 forms stable complex with Ras in-vitro:
it binds preferentially to nucleotide-free and
GTP-bound Ras

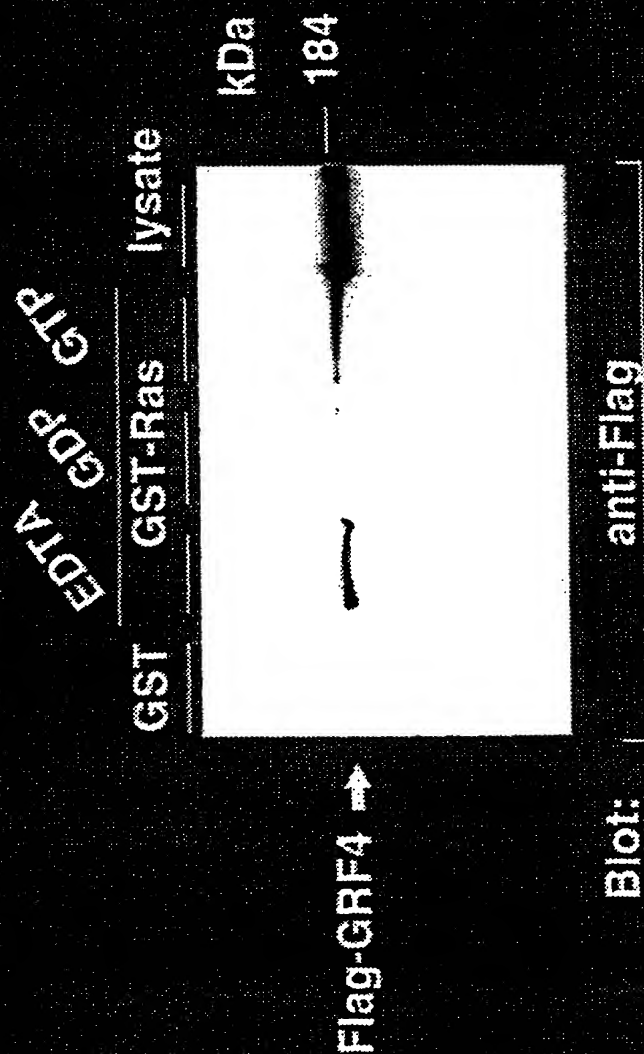


Figure 16

Transformation Assay:  GRF4 induces foci formation in Rat2 fibroblasts

RasV12

 GRF4

Empty Vector

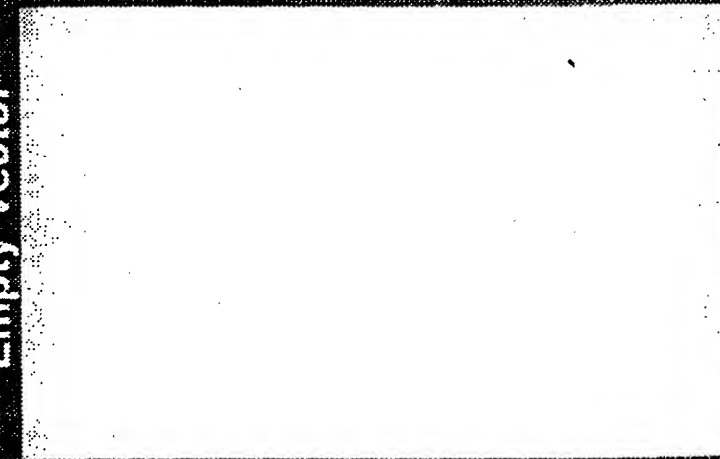
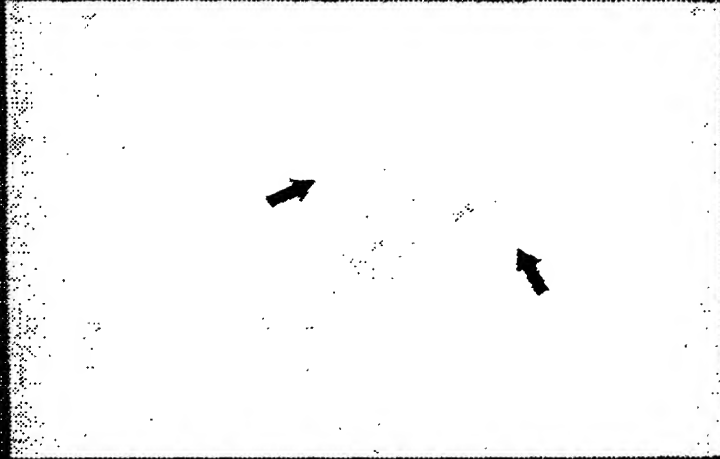
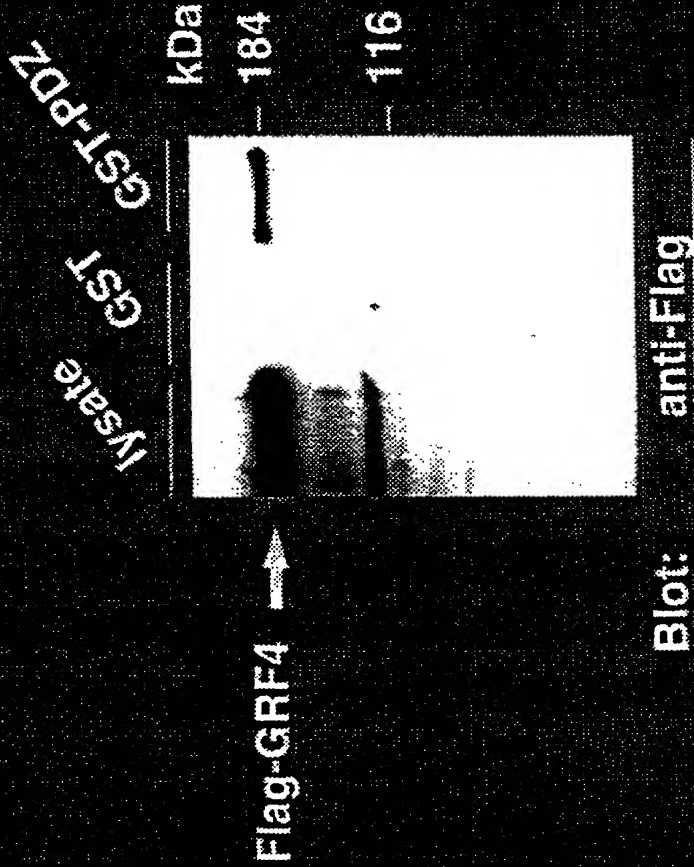


FIGURE 17

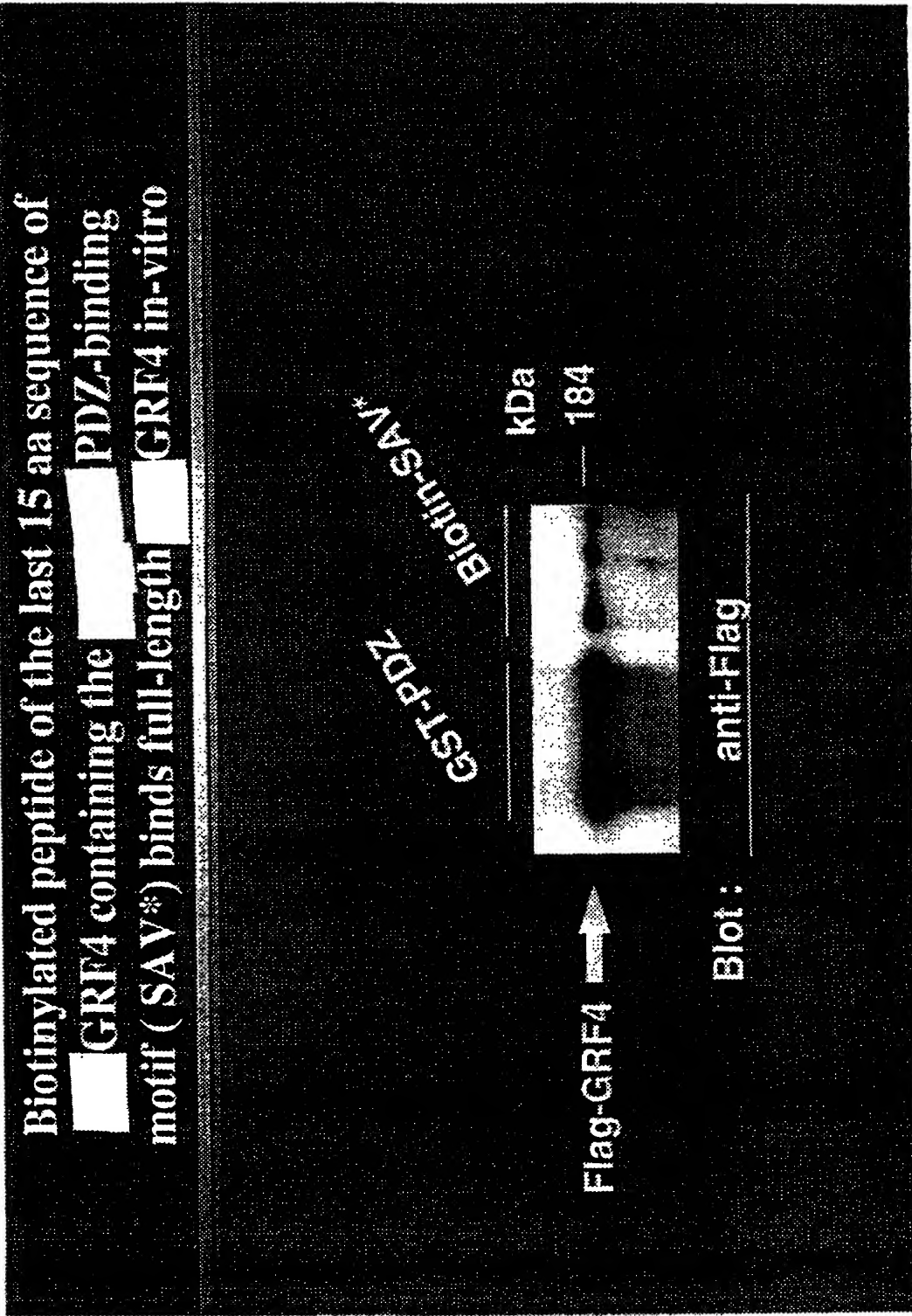
Figure 17

GST-fusion protein of  GRF4-PDZ
domain binds full-length  GRF4 in-vitro



T00220" 923TF600

Figure 18



6568 bp

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63. .4562

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121 cacttctcgc agatttcaca aaactgcatc ttactgacag tctccacca caggtgaccc
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(continued next page)

Figure 19A

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(continued next page)

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 6361 caattaactg gtgatttctt catactttg atactacttg tacctgtatg tcttttagaa
 6421 agacattggt ggagtctgta tccctttgt attttaata caataattgt acatattggt
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 6541 caagctggaa aataaataaa tataacat [SEQ ID NO: 1]

801 b.p.

1/1 31/11
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 L K G T K A G A P P R W R P L * N * W I
 * R E Q K L E L H R G G G R S R T S G S

61/21 91/31
 CCC CCG GGC TGC AGG AAT TGA AGC GGT GGG AAG GAT GTC TCC GCT GAG GCA GAG AGC AGC
 P P G C R N S S G G K D V S A E A E S S
 P R A A G I Q A V G R M S P L R Q R A A
 P G L Q E F K R W E G C L R * G R E Q Q

121/41 151/51
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 S M V P V T T E E A K P V P M P A H I A
 A W C P * L Q R K P N L S L C L P T * L
 H G A R D Y R G S Q T C P Y A C P H S C

181/61 211/71
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 * R R A L P R D S S H G R K A G T G S R
 D A E H Y Q G T H R T E G R Q V P G A A

241/81 271/91
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 P P T P P G Y V G I P I A D F P E G P C
 L P H L Q A T W A S P L P I S Q K G L A
 S H T S R L R G H P H C R F P R R A L P

301/101 331/111
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 T R P G S P R I T T W P C S G P A W W H
 P G Q E A P G L Q R G P A A V P H G G T

361/121 391/131
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 G P L R P R H R A R R R L Q P Q P A G R
 A H * G P G T G P D A A C S R S Q P A G

421/141 451/151
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 A A S H S G T S P A T Q T H A S R P S S
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 A G F A G A E E D E D E Q V S A V * G A

541/181 571/191
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 Q A P * S T V S H P K E S T R R R P K P
 R L L D P Q * A T Q R R A Q E D V P S L
 G S L I H S E P P K G E H K K T S Q A L

601/201 631/211
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 W S L G T H I * G W W T S L P P S L P *
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 E P W H A H L R M V D Q F A S F P A L K

661/221 691/231
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 Q H G A S S P L L P F P F A C E I L * R

(continued next page)

28/34

Figure 19B

721/241

751/251

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 K L P W H F A D L L L E M H S P A A P E
 N C P G T L Q T C C L K C T A Q Q P L S

781/261

GCT GCT GCC TGC CAC GTC ACG [SEQ ID NO: 3]
 A A A C H V T [SEQ ID NO: 4]
 L L P A T S [SEQ ID NO: 5]
 C C L P R H [SEQ ID NO: 6]

The PDZ domain (but not the C terminal SxV motif)
is required for plasma membrane localization of
GRF4

GRF4 Localization

WT



-PDZ



-SaV

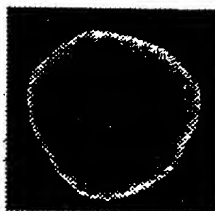


Figure 20

[illegible]

110323 33466

Figure 22

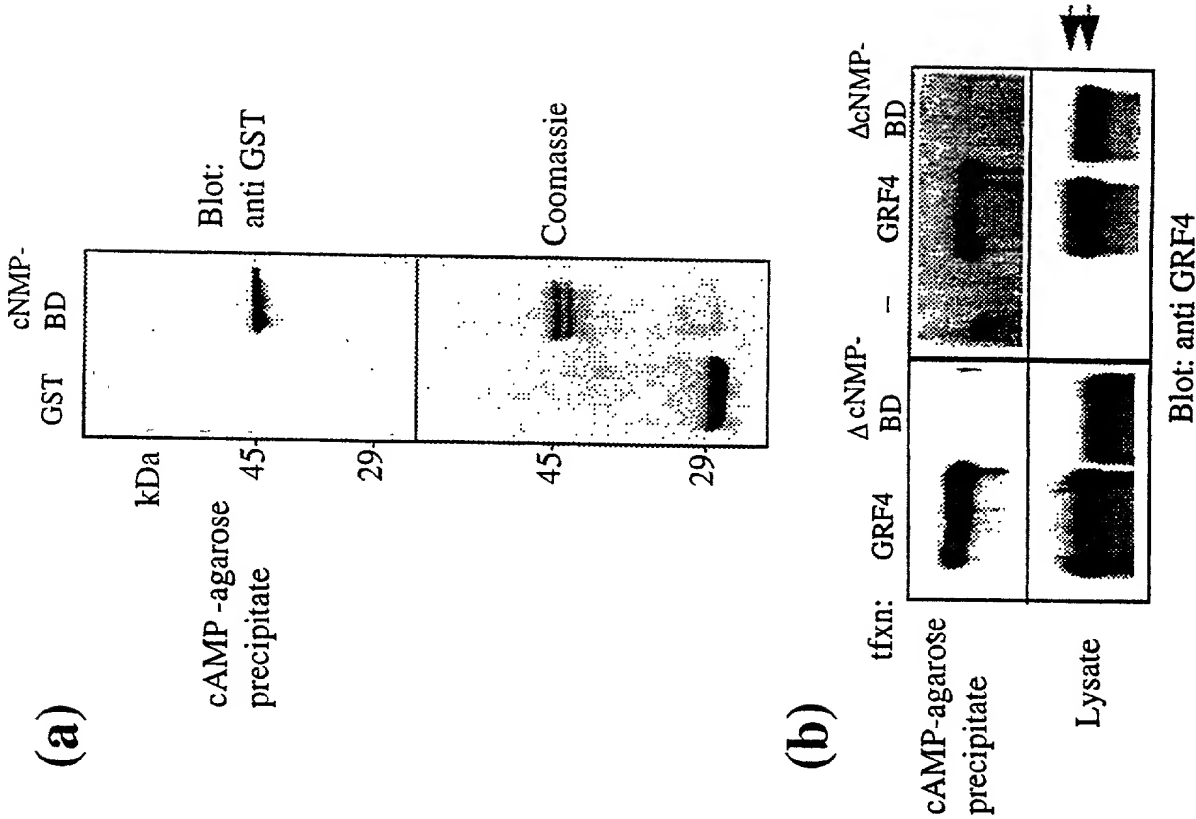
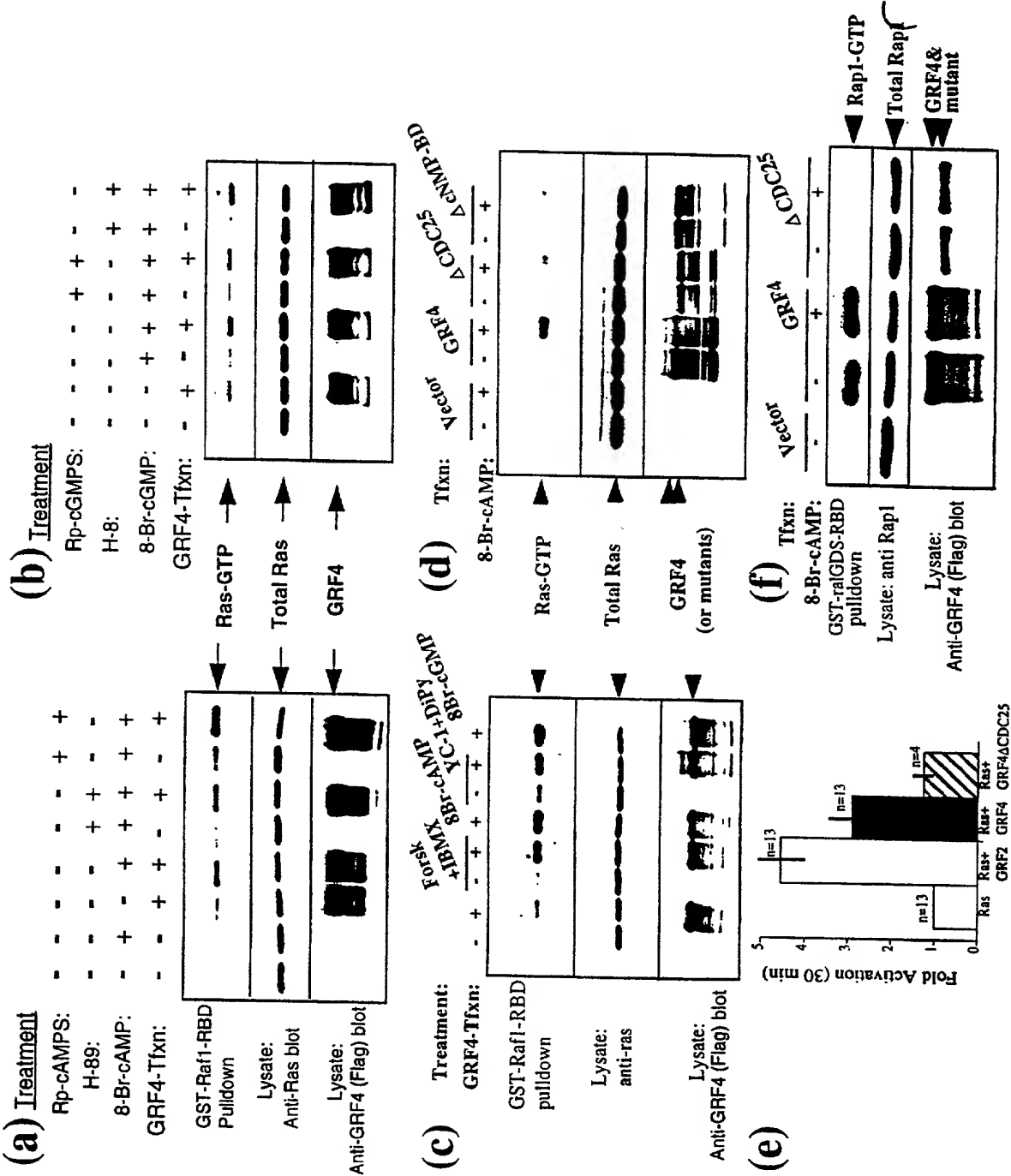


Figure 23



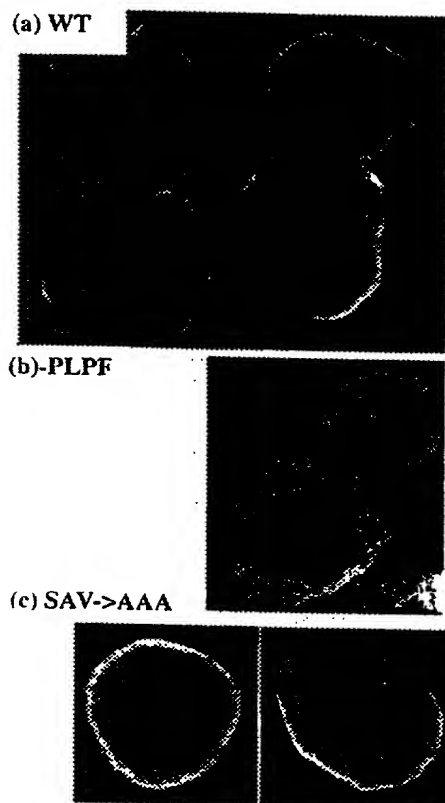


Figure 24

34/34